Organic carbon causes interference to nitrate and nitrite measurements by UV/Vis spectrometers : The importance of local calibration

Uusheimo, Sari Anneli

2017-07-27

Uusheimo , S A , Tulonen , T V , Arvola , L M J , Arola , H , Linjama , J & Huttula , T 2017 , ' Organic carbon causes interference to nitrate and nitrite measurements by UV/Vis spectrometers : The importance of local calibration ' , Environmental Monitoring and Assessment , vol. 189 , no. 357 , pp. 357 . https://doi.org/10.1007/s10661-017-6056-6

http://hdl.handle.net/10138/308890
https://doi.org/10.1007/s10661-017-6056-6

acceptedVersion

Organic carbon causes interference with nitrate and nitrite measurements by UV/Vis spectrometers: The importance of local calibration

Uusheimo, Sari[1]
sari.uusheimo@helsinki.fi
Phone: +358 50 4620555

Tulonen, Tiina[1]

Arvola, Lauri[1]

Arola, Hanna[2]

Linjama, Jarmo[3]

Huttula, Timo[2]

[1] Lammi Biological Station, University of Helsinki, Pääjärventie 320, FI-16900 Lammi, Finland

[2] Finnish Environment Institute, Freshwater Center, Survontie 9 A, FI-40500, Jyväskylä, Finland

[3] Finnish Environment Institute, P.O. Box 141, FI-00251 Helsinki, Finland

Abstract

Compared with sporadic conventional water sampling, continuous water-quality monitoring with optical sensors has improved our understanding of freshwater dynamics. The basic principle in photometric measurements is the incident light at a given wavelength that is either reflected, scattered, or transmitted in the body of water. Here, we discuss the transmittance measurements. The amount of transmittance is inversely proportional to the concentration of the substance measured. However, the transmittance is subject to interference, because it can be affected by factors other than the substance targeted in the water. In this study, interference with the UV/Vis sensor nitrate plus nitrite measurements caused by organic carbon was evaluated. Total or dissolved organic carbon as well as nitrate plus nitrite concentrations were measured in various boreal waters with two UV/Vis sensors (5-mm and 35-mm pathlengths), using conventional laboratory analysis results as references. Organic carbon increased the sensor nitrate plus nitrite results, not only in waters with high organic carbon concentrations, but also at the lower concentrations (< 10 mg C L^{-1}) typical of boreal stream, river, and lake waters. Our results demonstrated that local calibration with multiple linear regression, including both nitrate plus nitrite and dissolved organic carbon, can correct the error caused by organic carbon. However, high-frequency optical sensors continue to be excellent tools for environmental monitoring when they are properly calibrated for the local water matrix.

Keywords
Boreal water monitoring, calibration, nitrate, high-frequency sensor, optical measurement, organic carbon

Introduction

Excess nitrogen (N) has become one of the world´s main environmental challenges during recent decades (Rockström et al. 2009). Humans have doubled the amount of reactive nitrogen (Nr) on Earth mainly due to food production, wastewater, and fossil-fuel combustion (Gruber and Galloway 2008; Fowler et al. 2013), resulting in increased eutrophication and greenhouse-gas emissions, acid rain, smog, and stratospheric ozone depletion (Galloway et al. 2013). In agricultural watersheds and other nonpoint Nr sources, continuous monitoring of Nr is crucial to measuring N loading more accurately in dynamic water systems. Nutrient loading calculations based on traditional sporadic sampling generate uncertainty and may lead to under- or overestimations, especially in small lotic systems where spatiotemporal variation in water quality can be significant (e.g. Linjama et al. 2009; Koskiaho et al. 2010). Thus, high-frequency measurements are needed to better understand the relationships between land use, weather, and water chemistry in aquatic systems, especially in changing climates.

Optical sensors have a history spanning several decades in oceanographic and coastal studies, but their use in freshwater systems has increased only during the last decade (Pellerin and Bergamaschi 2014). One of the *in-situ* photometric sensors used is the ultraviolet/visible light (UV/Vis) scanning spectrolyzer manufactured by Messtechnik GmbH (Klagenfurt am Wörthersee, Austria). It is a multiparameter instrument that records the complete absorbance spectrum at wavelengths between 220 nm and 720 nm and is able to measure nitrate plus nitrite N (referred together as NO_x-N), dissolved organic carbon (DOC), total organic carbon (TOC), and turbidity. Sensors with distinct measuring pathlengths perform differently, because their precision and detection limits vary (spectrometer probe manual at www.s-can.at). Increased use of optical *in-situ* sensors has raised questions about their reliability, compared with the data obtained with conventional laboratory analyses.

For optical sensors with large absorbance spectra, it is not possible to differentiate the amounts of nitrate and nitrite, so they indicate the sum by the use of NO_x-N. The absorbance range of NO_x -N and organic carbon (OC) partly overlap, since they both absorb UV radiation, mainly at wavelengths between 200 nm and 250 nm (Van den Broeke et al. 2006). For the NO_x-N results, the interference likely comes from elements that have absorbance properties similar to those of OC, such as humic or fulvic acids, or inorganic elements such as bromide and hydrogen sulfide (Pellerin et al. 2013). Turbidity influences the entire absorption spectrum by shading and scattering light from suspended particles (Huber and Frost 1998). The effect of these substances can decrease the transmittance of light in the sample and result in overestimation of the NO_x-N concentration.

Manufacturers of optical sensors may offer various calibration options, depending on the application, without the absolute need for local calibration. The main purpose of these calibration options is to separate the NO_x-N signal from that of the interfering substances that typically occur in the types of waters studied (Pellerin et al. 2013). However, if differences with the reference method are observed, manufacturers recommend that local calibration be applied to enhance the reliability of the sensor results. One of the methods most used in calibration is simple linear regression (SLR); the results obtained from this reference method, usually in the laboratory, are plotted against the sensor results, and the linear function obtained is then applied to correct the sensor results (Huotari and Ketola 2014).

In this study, OC interference with the NO_x-N results was investigated with UV/Vis s:can spectrolyzer sensors having two different optical measuring pathlengths (35 mm and 5 mm). The TOC, DOC, and NO_x-N concentrations were analyzed with both sensors, as well as with laboratory methods for reference. To obtain a thorough water matrix for testing the performance of the sensors, we analyzed freshwaters ranging from spring water to a bog outlet and streams draining from agriculturally influenced catchments. We also spiked the spring and bog water samples with potassium nitrate (KNO_3) to obtain high NO_x-N concentrations. Finally, we applied the 5-mm pathlength sensor in an agricultural stream for 6 months to compare with the weekly collected samples analyzed in the laboratory. This information was used to consider the importance of OC in the local calibration method. This study provided much needed information regarding the local calibration of the UV/Vis sensor used in the NO_x-N measurements in freshwaters with high OC concentrations.

Materials and Methods

Experimental design

Various surface waters were collected from eight sites located in southern Finland for the two experiments. Samples for Experiment I were collected in 2009 (DOC analysis) and 2011 (TOC analysis). For Experiment II, the waters were collected in 2009. The water samples were kept in the dark and cold (+4 ˚C) until determined within a few hours of sampling by standard analytical methods and by the UV/Vis optical sensors. The laboratory and sensor measurements were performed simultaneously. To obtain a wide variety of combinations of NO_x-N and OC concentrations, the waters were mixed and/or diluted with deionized water (MQ) (Millipore; EMD Millipore, Billerica, MA, USA). Before the experiments, the accuracy of the OC measurements by the sensors was evaluated with laboratory TOC and DOC as references.

Measurements done in the laboratory with standard analytical methods conducted at Lammi Biological Station, University of Helsinki (see Arvola et al. 2015) were used as references for the sensor results. In this study, both TOC and DOC were considered as corresponding to OC. TOC was measured from unfiltered and DOC from filtered (< 0.2 µm, Nuclepore; Whatman GE Healthcare, Chicago, IL, USA) sample water, using a high-temperature combustion method with a TOC-5000 A analyzer (Shimadzu Corp., Kyoto, Japan). The detection limit of the TOC and DOC analyses varied between 0.2 and 1.0 mg L^{-1}, depending on the type of sample (e.g. turbidity). NO_x-N was analyzed from filtrates (< 0.2 µm, Nuclepore) with an automated flow-injection analyzer (Lachat QuikChem 8000 FIA; Lachat Instruments, Hach Company, Loveland, CO, USA), using a nitrate-reducing method with a cadmium column. The detection limit for the NO_x-N analysis was 0.01 mg L^{-1}.

The sensor NO_x-N measurements in the experiments were done with UV/Vis scanning spectrolyzers (Messtechnik) with 35-mm and 5-mm measuring pathlengths. The specifications state that the 35-mm pathlength sensor is capable of measuring NO_x-N concentrations < 10.0 mg L^{-1}. For TOC and DOC, the measurement ranges are below 25 and 12 mg C L^{-1}, respectively. At higher concentrations, the 5-mm pathlength sensor was more applicable, with measuring ranges for NO_x-N < 70 mg L^{-1}, for TOC < 150 mg C L^{-1}, and for DOC < 90 mg C L^{-1} (specifications at www.s-can.at). A specific cuvette designed for the sensors was mounted, and the sample was poured into the cuvette. Four to five

sequential readings were recorded for each sample. The cuvette was rinsed with MQ and sample water between measurements.

Table 1. Sampling sites, coordinates, water type, and mean NO_x-N, DOC, and TOC concentrations measured in the laboratory. The standard deviations are shown in parentheses if available. Not determined = *nd.*

Site	Coordinates	Type	NO_x-N mg L^{-1}	DOC mg L^{-1}	TOC mg L^{-1}
Laaviosuonoja Brook	61°2'N, 24°59'S	small peatland stream, high organic matter content	0.040 *(±0.010, n=6)*	74.0 *(±0.38, n=2)*	53.0 *(±0.61, n=3)*
Lake Ormajärvi	61°5'N, 24°57'S	medium-sized clear-water lake, mesotrophic	0.35 *(±0.010, n=3)*	*nd*	7.8 *(±0.030, n=3)*
Löytynlähde Spring	61°2'N, 24°58'S	spring, oligotrophic	0.41 *(±0.010, n=4)*	1.2 *(±0.29, n=2)*	2.3 *(±0.060, n=3)*
Luhtaanmäenjoki River	60°20'N, 24°47'S	small river, high turbidity, clay soils, agriculture	0.57	7.6 *(±0.037, n=2)*	*nd*
Lake Pääjärvi	61°5'N, 25°5'S	medium-sized humic lake, meso-oligotrophic	0.85	10.0 *(±0.038, n=2)*	*nd*
Teuronjoki River	61°5'N, 24°50'S	outflow river of Lake Pääjärvi	1.2	8.1 *(±0.033, n=2)*	*nd*
Vantaanjoki River	60°40'N, 24°56'S	small river, high turbidity, clay soils, agriculture	1.3 *(±0.012, n=3)*	*nd*	10.0 *(±0.24, n=3)*
Letkunoja Brook	61°3'N, 25°5'S	small stream, mineral soils, agriculture	2.5	7.9 *(±0.028, n=2)*	*nd*
Koiransuolenoja Brook	61°3'N, 25°4'S	small stream, mineral soils, agriculture	2.4 *(±0.37, n=25)*	10.6 *(±4.5, n=25)*	*nd*

Experiment I: In the first part of the experiment in 2009, water samples were collected from six sites (Laavionsuonoja, Löytynlähde, Teuronjoki, Letkunoja, Luhtaanmäenjoki, and Pääjärvi (Table 1). In addition, one mixed sample (1:1) of two streams (Laavionsuonoja : Luhtaanmäenjoki) was prepared. After analyzing the samples in the laboratory for NO_x-N and DOC, dilutions with these seven samples and MQ were done at ratios of 1:1, 1:3, 1:9, and 3:1. The diluted concentrations were calculated based on the laboratory-analyzed initial concentrations and compared with the sensor results. In the second part of the experiment, water samples were collected in 2011. Three to six replicate water samples from each of four sites (Laavionsuonoja, Löytynlähde, Ormajärvi, and Vantaanjoki) were analyzed in the laboratory for NO_x-N and TOC before making the dilutions and mixtures presented in Table 2. The concentrations were analyzed in the laboratory and compared with the sensor results.

Table 2. Mixtures of the waters examined in Exp. I from the 2011 samples (only TOC analyzed). MQ = deionized water.

Sample	Mixing Ratios
Laavionsuonoja: Vantaanjoki	1:1
Löytynlähde: Vantaanjoki	2:1
Laavionsuonoja: Löytynlähde	1:2, 1:4
Vantaanjoki: Ormajärvi	1:1, 1:2
Ormajärvi: MQ	1:1, 1:2, 1:3, 1:4
Löytynlähde: MQ	1:1
Löytynlähde: Ormajärvi: MQ	1:1:1

Experiment II (only the 5-mm pathlength sensor): In this experiment, KNO_3 was added to waters collected from Laavionsuonoja and Löytynlähde, to determine the lower and upper detection limits for NO_x-N in the presence of high and low OC concentrations. First, the NO_x-N concentrations were analyzed in the laboratory from the original samples. The sample waters were spiked with KNO_3 (1000 mg N L^{-1}), and the NO_x-N concentrations were: Laavionsuonoja 0.53, 2.03, 8.03, 16.0, and 32.0, and Löytynlähde 1.6, 2.6, 8.6, 16.6, and 32.6 mg N L^{-1}. Based on the known initial NO_x-N concentrations, the calculated values of NO_x-N were compared with the corresponding sensor results.

Experiment III: The 5-mm pathlength sensor measured NO_x-N and DOC in Koiransuolenoja for 6 months from early May to late November in 2013. These data were compared with weekly collected samples analyzed in the laboratory. The sensor data were calibrated in two different ways: simple linear regression (SLR) and multiple linear regression (MLR). In SLR, the calibration was based on the regression function between the laboratory and sensor NO_x-N. In MLR, the data calibration was based on the sensor NO_x-N and sensor DOC results in relation to the laboratory NO_x-N results, and the sensor NO_x-N data were corrected, based on the resulting regression function. This information was used to demonstrate the impact of OC on the local calibration procedure.

Data analysis

SLR was applied to compare the laboratory (x-axis) and sensor results (y-axis). The accuracy of the sensor results was evaluated by the correlation coefficient (R^2) of the regression function. Due to the rather small sample sizes and the skewness of the data, statistical differences between the sensor and laboratory results were investigated with the nonparametric Wilcoxon signed-ranks test. The level of statistical significance was 0.05. The data were analyzed in their original form to maintain the relationship between the laboratory and sensor data. The effect of the increasing DOC concentration on the NO_x-N sensor results was evaluated by the proportional difference between the laboratory and sensor results (referred to as the NO_x-N error ratio). If the DOC concentration had not affected the NO_x-N results by the sensor, the error ratio would have been steady. In Experiment III, we applied two different local calibration methods: SLR and MLR, and analyzed their accuracy by comparing the laboratory results with the corrected sensor data in linear regression and nonparametric Wilcoxon signed-ranks test. Data analyses were performed, using Microsoft

Excel for Mac (15.33; Microsoft Corp., Redmond, WA, USA) and IBM SPSS Statistics for Macintosh version 24.0 (IBM Corp., Armonk, NY, USA).

Results

The water quality varied between the sampling sites (Table 1.). The lowest NO_x-N concentration was detected in the bog outlet (Laavionsuonoja) and the highest were found in the brook and river waters draining from the agriculturally influenced catchments. Respectively, the lowest concentration of OC was measured in Löytynlähde and the highest in Laavionsuonoja.

The laboratory-measured TOC and DOC concentrations in the experimental waters varied between 1.3-53.0 and 0.12-74.0 mg C L^{-1}. Two TOC concentrations (31.0 and 53.0 mg C L^{-1}) and seven DOC concentrations (19-74.0 mg C L^{-1}) were out of the measurement range of the 35-mm pathlength sensor (TOC < 25 mg C L^{-1} and DOC < 12 mg C L^{-1}), and these were omitted from the analysis. Additionally, the 35-mm pathlength sensor was unable to measure the TOC in one sample from Laavionsuonoja, whereas the laboratory-analyzed TOC was 19.0 mg C L^{-1}. The one outlier seen in Fig.1a was a sample from Laavionsuonoja. All the DOC/TOC concentrations were within the measuring range of the 5-mm pathlength sensor. A strong linear relationship ($p < 0.001$) was found between both sensors and the laboratory OC results, but the sensor values were significantly (22-29%) higher than the laboratory results (**Fig. 1**) (35-mm: Z = -3.269, sig. = 0.001; 5-mm: Z = -4.462, sig. = 0.000).

Fig. 1a, b Laboratory TOC/DOC concentrations in relation to measured concentration by a) 35-mm pathlength sensor and b) 5-mm pathlength sensor

Experiment I

The laboratory-analyzed and -calculated NO_x-N concentrations varied between 0.010 and 3.8 mg N L^{-1} (n = 63). Thus, the concentrations settled within the measurement range for both of the sensors given by the manufacturer in the specifications.

For the 35-mm pathlength sensor, a strong linear correlation ($R^2 = 0.88$, $p < 0.001$, n = 40) was evident between the sensor and laboratory NO_x-N results in samples with OC concentrations of 1.3-10.0 mg C L^{-1} (TOC) and 0.12-10.0 mg C L^{-1} (DOC). The linear relationship strengthened when the bog water samples (n = 3) were removed from the regression analysis ($R^2 = 0.93$) (**Fig. 2a**). However, the sensor indicated 26% higher NO_x-N concentrations than did the laboratory results with statistical significance (Z = -1.989, sig. = 0.047). The sensor was not able to measure NO_x-N in two bog water samples and gave 'NaN' (Not a Number) indications. The NO_x-N and TOC concentrations in these samples were 0.23, 0.28 mg N L^{-1} and 19.0, 13.0 mg C L^{-1}, respectively.

For the 5-mm pathlength sensor, the OC concentration in all the samples (n = 51) fell inside the measurement range of the sensor (TOC < 150 and DOC < 90 mg C L^{-1}.) However, the linear dependency between the NO_x-N sensor and laboratory results was weak ($R^2 = 0.024$, $p < 0.28$). Use of the sensor resulted in significant overestimation, compared

with the laboratory results (Z = -2.925, sig. = 0.003), due to the bog outlet waters from Laavionsuonoja having high OC concentrations (TOC 13.0-53.0 mg C L^{-1}, DOC 4.0-74.0 mg C L^{-1}) and low NO_x-N concentrations. Omission of these bog water samples from the analysis significantly increased the linear dependency ($R^2 = 0.96$, $p < 0.001$, $n = 37$). Yet, the sensor resulted in overestimation of the NO_x-N concentration by 32% compared with the laboratory results, but the difference was not verified statistically (Z = -0.228, sig. = 0.820).

Fig. 2a, b Relationships of NO_x-N between the sensor results and laboratory measurements at DOC/TOC ranges of 0.12-10.0 mg C L^{-1}. The bog outlet waters from Laavionsuonoja (marked with empty circles) were excluded from the regression lines. The detection limit for the laboratory NO_x-N was 0.010 mg N L^{-1}

Low NO_x-N concentrations

The results showed that both sensors experienced difficulty when samples contained low levels of NO_x-N (0.010-0.43 mg NO_x-N L^{-1}). As shown in **Fig. 3a**, the NO_x-N concentrations in samples containing Laavionsuonoja water ($n = 3$, DOC 4.03-10.0 mg C L^{-1}) were highly overestimated (10-50 times higher than the laboratory result), using the 35-mm pathlength sensor. No strong linear dependency between the sensor and laboratory results could be observed ($R^2 = 0.59$, $p = 0.000$). In addition, eight samples with NO_x-N concentrations between 0.056 and 0.28 mg N L^{-1} resulted in readings of zero by the sensor. The OC concentrations in these samples were low (TOC 1.8-2.1 and DOC 0.12-0.99 mg C L^{-1}). However, the inability to measure NO_x-N concentrations < 0.28 mg N L^{-1} was not consistent, since the sensor was able to measure 10 other samples < 0.28 mg N L^{-1} (TOC 1.3-4.0, DOC 0.79-7.4 mg C L^{-1}).

The 5-mm pathlength sensor showed a higher measurement range for OC. Thus, it was able to measure more samples containing bog water within the range 0.010-0.43 mg NO_x-N L^{-1} (**Fig. 3b)**. However, the NO_x-N concentrations measured by the sensor were overestimated by a greater extent (5-130 times higher than the laboratory result). No strong linear dependency could be observed between the laboratory and sensor results, even without the bog water samples ($R^2 = 0.55$, $p = 0.000$). Similarly, difficulty in measuring low NO_x-N concentrations was observed, since the sensor recorded no results in 12 samples with NO_x-N concentrations of 0.056-0.28 mg L^{-1}. The OC concentrations in these samples were 1.3-2.7 mg C L^{-1} as TOC and 0.12-1.9 mg C L^{-1} as DOC. Yet, the sensor was able to measure 16 other samples with NO_x-N concentrations < 0.28 mg L^{-1}, of which 13 were bog water samples (TOC 3.3-53.0, DOC 2.5-74.0 mg C L^{-1}).

Fig. 3a, b Relationship for the NO_x-N concentrations 0.010 – 0.43 mg L^{-1} by the sensors, compared with the laboratory results: a) 35-mm pathlength sensor, b) 5-mm pathlength sensor. The bog outlet waters from Laavionsuonoja (empty circles) are omitted from the regression lines. The detection limit (0.010 mg N L^{-1}) of the laboratory NO_x-N is indicated by the dashed line. Note the different scales

Experiment II

The initial laboratory-analyzed NO_x-N concentrations in Laavionsuonoja and Löytynlähde were 0.031 and 0.56 mg NO_x-N L^{-1}, respectively. The DOC concentration in Löytynlähde was 0.12 mg C L^{-1} and in the bog outlet water of Laavionsuonoja 74.0 mg C L^{-1}. All the concentrations were within the measurement range given by the manufacturer of the 5-mm pathlength sensor (NO_x-N < 70.0 mg N L^{-1} and for DOC < 90.0 mg C L^{-1}).

The 5-mm pathlength sensor failed to measure NO_x-N in the two highest concentrations, 16.0 and 32.0 mg NO_x-N L^{-1} (**Fig. 4)** and gave 'NaN' indications in the high-OC waters of Laavionsuonoja. As a result, the measurement range observed was 0.031-8.03 mg NO_x-N L^{-1}, having a strong linear correlation ($R^2 = 0.99$, $p < 0.01$). In the spring water with low OC, the sensor measured all the samples accurately ($R^2 = 1.0$, $p < 0.001$) within the concentration range 0.56-33.0 mg NO_x-N L^{-1}.

Fig. 4 NO_x-N concentrations of Laavionsuonoja (empty circles) and Löytynlähde (filled circles) measured with the 5-mm pathlength sensor, compared with the laboratory results. The detection limit for the laboratory NO_x-N was 0.010 mg N L^{-1}

NO_x-N error ratio

The proportional difference between the laboratory and sensor results is referred to as the NO_x-N error ratio. This ratio was plotted against the corresponding DOC concentration to demonstrate the effects of OC on the NO_x-N results by the sensors. Only study sites with at least four data points were included. Based on the results, the NO_x-N error ratio for the 35-mm pathlength sensor mostly increased along with the corresponding DOC concentration (DOC < 10.0 mg C L^{-1}), yet remained below 2.0 (**Fig. 5**). The OC strongly influenced the NO_x-N results by the 5-mm pathlength sensor in the bog waters, resulting in error ratios of 40.0-66.0 and 3.7-15.0 (**Fig. 6a**). The first data points in these lines (error ratios 40.0 and 3.7) were observed in DOC concentrations of 7.4 and 4.03 mg C L^{-1}. In samples with no bog waters (DOC below 10.0 mg C L^{-1}), the NO_x-N error ratio remained under 2.0 (**Fig. 6b**).

Fig. 5 NO_x-N error ratios for the 35-mm pathlength sensor with corresponding DOC concentrations (< 10.0 mg C L^{-1}) in Letkunoja, Teuronjoki, Pääjärvi, and Luhtaanmäenjoki

Fig. 6a, b NO_x-N error ratios for the 5-mm pathlength sensor with corresponding DOC concentrations, including a) bog outlet waters with high levels of OC (Laavionsuonoja and mixture of Laavionsuonoja and Luhtaanmäenjoki) and b) Letkunoja, Teuronjoki, and Pääjärvi. Note the different scales

Experiment III

Sensor data collected from Koiransuolenoja was corrected with two different methods. In SLR, the correlation coefficient of the regression function between the sensor and laboratory NO_x-N was weak ($R^2 = 0.32$, $n = 25$). In MLR, the sensor NO_x-N data were corrected, based on the resulting regression function (laboratory NO_x-N = 781.087 - (74.994 * scan-DOC) + (0.935 * scan-NO_x-N), and the model was significant ($R^2 = 0.92$, $p = 0.000$). Yet, both sensor results showed the dynamic nature of the NO_x-N concentration in the agriculturally influenced stream. As can be seen in **Fig. 7a** and **b**, the sensor data corrected by MLR were more accurate ($R^2 = 0.93$, $p = 0.000$) than the sensor data with higher variability corrected by SLR ($R^2 = 0.33$, $p < 0.010$). The mean NO_x-N concentrations were similar with both correction methods (2.4 mg L^{-1}), and no statistical differences were found in the medians with the Wilcoxon signed-ranks test.

Fig. 7a, b Laboratory NO_x-N results indicated with circles (n = 25) in relation to the continuous 5-mm pathlength sensor results from Koiransuolenoja Brook on 3 May to 22 November 2013 corrected by a) simple linear regression (laboratory NO_x-N result as the only explanatory variable) and b) multiple linear regression (explanatory variables scan-DOC, scan-NO_x-N) (unpublished data, Uusheimo et al.)

Discussion

Originally, optical nitrate sensors were developed for ocean environments with low turbidity and color (Pellerin et al. 2013). Thus, their use in various aquatic environments, such as rivers and lakes with high turbidity and color, calls for careful planning in application procedure. Use of these sensors resulted in increasing overestimation of the NO_x-N concentrations under ascending OC, which was not a result of inaccurate measurement of OC, but rather due to the partial absorption of light at the same wavelengths. The 35-mm pathlength sensor, recommended for lower NO_x-N and OC concentrations, performed according to the manufacturer´s specifications, excluding the samples of bog outlet waters, in which the TOC concentrations fell within the measuring range given by the manufacturer. This result is in agreement with the findings of Drolc and Vrtovšek (2010), who observed that the NO_x-N values obtained from the sensor were higher than those from the reference method, due to interference from other substances in the local water matrix. Additional support for cross-sensitivity between OC and NO_x-N was found in the KNO_3 amendment experiment, in which the measuring range of NO_x-N by the 5-mm pathlength sensor was clearly decreased under the influence of the high OC levels in the bog outlet water. However, a matter of OC quality was also seemingly involved, because the NO_x-N error ratio was higher in the bog outlet waters than in the river, stream, or lake water. It should be noted that the error ratio was not linear. In addition, both sensors were unreliable at measuring low NO_x-N concentrations (< 0.28 mg N L^{-1}) often resulting in readings of zero in the spring, lake and stream waters. This should be considered, especially in working with waters typically low in nitrates, such as forest areas, oligotrophic lakes, headwaters, and other more pristine areas.

As the manufacturer states, the accuracy of the measurements can be improved by local calibration, i.e. correcting the sensor results with sufficient manual sampling. Caradot et al. (2015) suggested that calibration should be based on at least 15-20 samples, covering for the most part the variation in concentration of the substance measured. There are several procedures for calibration; Lepot et al. (2016) used SLR, which has proven a robust and applicable tool. Our results show that if sufficient accuracy in sensor NO_x-N results is not achieved by simple correction, an MLR including OC data can be applied to achieve a more accurate correction.

Based on the results of this study, local calibration in NO_x-N measurements with optical sensors should always be carried out in dealing with waters containing some organic matter. Streams, lakes, and other natural boreal waters can contain high amounts of OC. In Finland, due to the large areas of coniferous forest and marshland, the average TOC of surface waters ranges from 0.50 to 47.0 mg L^{-1} (median 12.0 mg L^{-1}) (Kortelainen 1999). In boreal latitudes, seasonality may also result in changes in freshwater OC quantity and quality (e.g. Erlandsson et al. 2012). Interference of organic matter in NO_x-N measurements with optical sensors may become even more relevant, due to future changes in climate and their effects on OC.

In conclusion, neither sporadic manual sampling nor continuous sensor monitoring can ensure excellent data quality alone. Our results demonstrate that sensor data need to be verified with samples analyzed in the laboratory. When the data quality of photometric sensors has been assured with care, high-frequency measurements by sensors can provide extremely useful data and improve our knowledge of water quality and nutrient loading, especially in highly dynamic aquatic environments.

Acknowledgements

This study was funded by the M. and T. Nessling Foundation. We thank the laboratory staff of Lammi Biological Station for conducting the laboratory work. This study was conducted under projects: ERDF-funded New Solutions for Environmental Monitoring (YMLI, YMPANA), Profitability and Added-Value for Agriculture from Automatic, Real-Time Monitoring of Environment and the Extension Services (EnviSense), Measurement, Monitoring and Environmental Assessment (MMEA), Processes Controlling DOC-Fluxes in Boreal Catchments (PRO-DOC), and N-Sink (LIFE12 ENV/FI/597).

References

Arvola, L., Einola, E. & Järvinen, M. (2015). Landscape properties and precipitation as determinants for high summer nitrogen load from boreal catchments. *Landscape Ecology*. 30:429-442.

Caradot, N., Sonnenberg, H., Roualt, P., Gruber, G., Hofer, T., Torres, A., Pesci, M. & Bertrand-Krajewski, J-L. (2015). Influence of local calibration on the quality of online wet weather discharge monitoring: feedback from five international case studies. *Water Science & Technology* Vol 71(1), pp 45-51. IWA Publishing.

Drolc, A. & Vrtovšek, J. (2010). Case study: Nitrate and nitrite nitrogen determination in waste water using on-line UV spectrometric method. *Bioresource Technology*. 101:4228-4233.

Erlandsson, M., Futter, M.N., Kothawala, D.N. & Köhler, S.J. (2012). Variability in spectral absorbance metrics across boreal lake waters. *Journal of Environmental Monitoring* 14, 2643, doi:10.1039/c2em30266g

Fowler, D., Coyle, M., Skiba, U., Sutton, M., Cape, N., Reis, S., Sheppard, L. J., Jenkins, A., Grizzetti, B., Galloway, J. N., Vitousek, P., Leach, A., Bouwman, A. F., Butterbach-Bahl, K., Dentener, F., Stevenson, D., Amann, M. & Voss, M. (2013). The global nitrogen cycle in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences* 368:20130164.

Galloway, JN, Leach, AM, Bleeker, A, Erisman, JW. (2013). A chronology of human understanding of the nitrogen cycle. *Philosophical Transactions of the Royal Society B: Biological Sciences*, doi.org/10.1098/rstb.2013.0120

Gruber, N. & Galloway, J. N. (2008). An earth-system perspective of the global nitrogen cycle. *Nature*. Vol 451:293-296.

Huber, E. & Frost, M. (1998). Light scattering by small particles. *Aqua*, 47: 87–94. doi:10.1046/j.1365-2087.1998.00086

Huotari, J. & Ketola, M. (2014). Jatkuvatoiminen levämäärien mittaus. Hyvät mittauskäytännöt ja aineiston käsittely. *Ympäristöhallinnon ohjeita* 5/2014.

Kortelainen, P. (1999). Occurrence of humic waters. Temporal and spatial variability. In: J. Keskitalo, & P. Eloranta, (eds.). *Limnology of Humic Waters*. (pp. 46-55). Leiden, Backhuys Publishers.

Koskiaho, J., Lepistö, A., & Tattari, S. (2010): On-line measurements provide more accurate estimates of nutrient loading: a case of the Yläneenjoki river basin, southwest Finland. *Water Science & Technology*. Vol 62 No 1 pp 115–122 IWA Publishing.

Lepot, M., Torres, A., Hofer, T., Caradot, N., Grubber, G., Aubin, J-B & Bertrand-Krajewski J-L. (2016). Calibration of UV/Vis spectrophotometers: A review and comparison of different methods to estimate TSS and total and dissolved

390 COD concentrations in sewers, WWTPs and rivers. *Water Research* 101:519-534.
391
392 Linjama, J., Puustinen, M., Koskiaho, J., Tattari, S., Kotilainen, H. & Granlund, K. (2009). Implementation of
393 automatic sensors for continuous monitoring of runoff quantity and quality in small catchments. *Agricultural and Food*
394 *Sciences*. Vol 18:417-427.
395
396 Pellerin, B.A., Bergamaschi, B.A., Downing, B.D., Saraceno, J., Garret, J.D. & Olsen, L.D. (2013). Optical techniques
397 for the determination of nitrate in environmental waters: Guidelines for instrument selection, operation, deployment,
398 maintenance, quality assurance, and data reporting. *USGS Techniques and Methods Report* 1-D5, 37 pp.

399 Pellerin, B.A.& Bergamaschi, B.A. (2014). Optical Sensors for Water Quality. *Lakeline.* Spring 2014.

400 Rockstrom, J., W. Steffen, K. Noone, A. Persson, F. S. Chapin, III, E. Lambin, T. M. Lenton, M. Scheffer, C. Folke, H.
401 Schellnhuber, B. Nykvist, C. A. De Wit, T. Hughes, S. van der Leeuw, H. Rodhe, S. Sorlin, P. K. Snyder, R. Costanza,
402 U. Svedin, M. Falkenmark, L. Karlberg, R. W. Corell, V. J. Fabry, J. Hansen, B. Walker, D. Liverman, K. Richardson,
403 P. Crutzen, and J. Foley. (2009). Planetary boundaries: exploring the safe operating space for humanity. *Ecology and*
404 *Society* 14(2): 32. http://www.ecologyandsociety.org/vol14/iss2/art32/.

405 Van den Broeke, J., Langergraber, G. & Weingartner, A. 2006. On-line and in-situ UV/vis spectroscopy for multi-
406 parameter measurements: a brief review. *Spectroscopy Europe.* Vol 18:1-4.